Note

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# Biotransformation of Paclitaxel by Cell Suspension Cultures of *Marchantia polymorpha*

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**Abstract**

Biotransformation of paclitaxel by cell suspension cultures of *Marchantia polymorpha* was studied and it was found that liverwort cultures convert paclitaxel to 7-epi-paclitaxel in good yield and regioselectively hydrolyses the ester group at the 10-position of paclitaxel.

As paclitaxel (**1**), a complex diterpenoid isolated from the bark of *Taxus brevifolia* Nutt. (family Taxaceae) (Wani *et al.*, 1971), exhibits high activity against ovarian cancer, its biotransformation by mammalian and plant cells is of considerable interest. Monsarrat *et al.* studied biotransformation of paclitaxel by the rat (Monsarrat *et al.*, 1990) and it was found that rat liver converts paclitaxel to several taxoids such as 6-hydroxy-taxol. More recently we have reported on the epimerization of paclitaxel by human cancer cells (jurkat) (Hamada *et al.*,1996a) and regioselective hydrolysis of paclitaxel by plant cells of *Eucalyptus perriniana* (Hamada *et al.*,1996b).

We have investigated biotransformation of foreign substrate by *in vitro* cultured plant cells (Hamada *et al.*,1991a; Hamada *et al.*,1993; Hamada *et al.*,1994; Hirata *et al.*,1994; Hamada *et al.*,1994; Nakamura *et al.*,1995) and found that plant cell suspension cultures can perform regioselective hydroxylations, stereoselective reductions, and enantioselective oxidations. To investigate the metabolism of paclitaxel (**1**) by *Marchantia polymorpha* (liverwort) we study the biotransformation of **1** by liverwort cell suspension cultures. Here we report on the biotransformation of paclitaxel by cell suspension cultures of *Marchantia polymorpha* in which the liverwort cell suspension cultures convert paclitaxel (**1**) to 10-deacetylpaclitaxel (**2**) and to 7-epi-paclitaxel (**3**).

Cultured cells of *M. polymorpha* used in this study were induced and maintained as previously reported (Hamada *et al.*, 1991b). The cells had a green color and the growth of the cells were considerably rapid. The cells (30 g fresh weight) were subcultured into a 500-m*l* conical flask containing 150 m*l* of MSK-II medium (Katoh *et al.*, 1980) and then grown with continuous shaking for 1 week at 25 ℃ in continuous light (2000 lux). Paclitaxel (5 mg in 100 $\mu l$ of 3 kinds of solvent) was then administered to the flask and the cultures were continued under the same conditions. After 10 days, cells were removed by filtration and the culture medium was extracted with $CH_2Cl_2$. After evaporation of the solvent, the organic extract was subjected to HPLC analysis. HPLC was performed on a CrestPak C18S (4.6 mm x 150 mm) column using MeOH-$H_2O$ (65:35) as mobile phase with detection at 227 nm.

Chromatographic analysis showed that part of the exogenous paclitaxel (**1**) was converted to two compounds (**2** and **3**) (**Table 1**). These two compounds were obtained from medium extract.

Characterizations of products, **2** and **3** were carried out by HPLC-DAD (diode array detection) and LC-MS spectrometry (**2**:$[M+Na]^+$ 834; **3**: $[M+Na]^+$ 876). The structures of **2** and **3** were determined by interpretation of their $^1H$ NMR and MS spectra and comparison with reported data (Hamada *et al.*, 1996b); they were identified as 10-deacetyl-paclitaxel and 7-epi-paclitaxel, respectively. After 10-day incubation **1** was converted to **2** and **3**, 3.0% and, 2.2% respectively without solvent. In EtOH solvent **1** was converted to **2** and **3**, 4.7% and 53.6%, respectively. As shown in **Table 1** the conversion yield with solvent is much higher than that without solvent. This result indicated that **1** becomes incorporated easily into the liverwort cells. Also the yield of hydrolysis of the ester group at 10

**Table 1.** Biotransformation of paclitaxel by cell suspension culture of *M.polymorpha*

| Substrate | Solvent | Product yield (%)* | |
|---|---|---|---|
| | | 10-deacetylpaclitaxel (**2**) | 7-epi-paclitaxel (**3**) |
| Paclitaxel (**1**) | None | 3.0 | 2.2 |
| | Acetone | 2.2 | 52.2 |
| | EtOH | 4.7 | 53.6 |
| | DMSO | 3.6 | 43.7 |

* Weight (%) of product relative to the substrate administered.

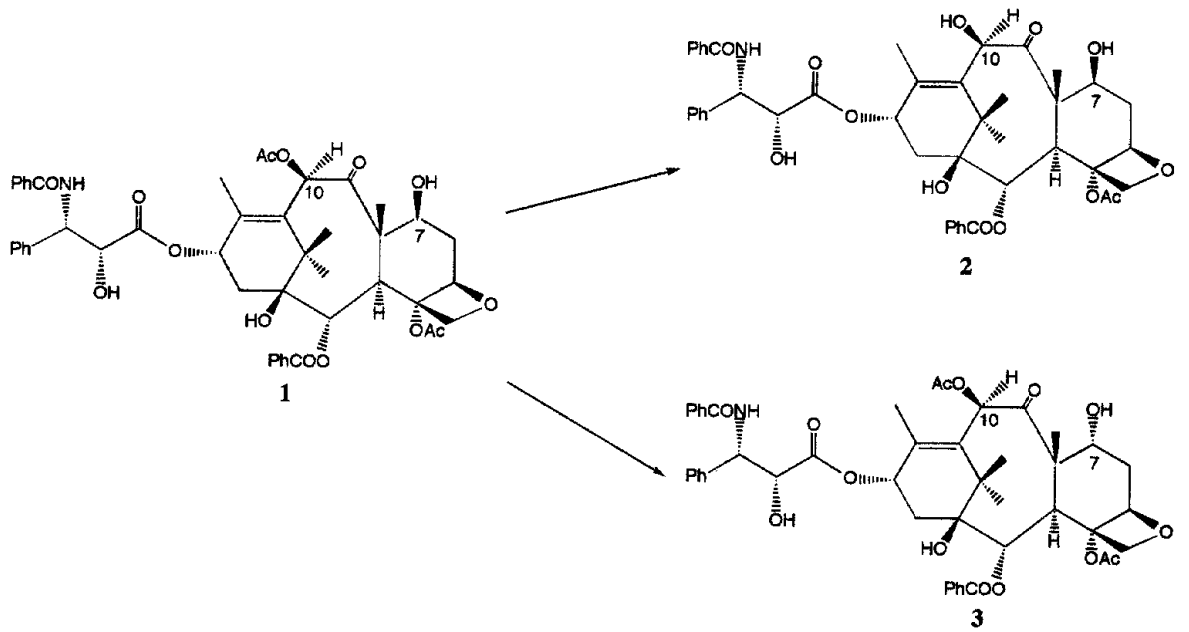


**Fig. 1** Biotransformation of paclitaxel (**1**) by the cultured suspension cells of *M.polymorpha*

-position of **1** is low. On the other hand the yield of epimerization at 7-position of **1** is high. From this result it can be assumed that cell suspension cultures of *M. polymorpha* more readily epimerize than hydrolyse **1**. The mechanism of the epimerization through a retro-aldol reaction is proposed by our previous paper (Hamada *et al.*, 1996a). As shown in **Fig. 1**, **1** was converted to **2** and **3**, independently. Careful and repeated TLC and HPLC analyses showed no other products. Incubation of paclitaxel in culture medium without *M. polymorpha* cells showed that paclitaxel was unchanged.

Thus, it was found that the suspension cultures of *M. polymorpha* catalyze the epimerization of paclitaxel to 7-epi-paclitaxel and regioselectively hydrolyze the ester group at 10-position of paclitaxel. The biotransformation of paclitaxel by cultured suspension cells of other plant species and by isolated enzymes is now in progress.

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## References

Hamada, H., Nakazawa, K., 1991a. Biotransformation of vinblastine to vincristine by cell suspension cultures of *Catharanthus roseus*. Biotechnology Letters, **12:** 805-806.

Hamada, H., Kawabe, S., 1991b. Biotransformation of 4-androstene-3,17-dione by green cell suspension of *Marchantia polymorpha*: Stereoselective reduction at carbon 17. Life Sciences, **48:** 613-615.

Hamada, H., Fuchikami, Y., Ikematsu, Y., Hirata, T., Williams, H.J., Scott, A.I., 1994. Hydoxylation of piperitone by cell suspension cultures of *Catharanthus roseus*. Phytochemistry, **37:** 1037-1038.

Hamada, H., Imura, M., Okano, T., 1994. Biotransformation of 1, 1, 1-trifluoroheptan-2-one and octan-2-one by the cell suspension cultures of *Nicotiana tabacum*-

Acceleration by fluorine substitution. Journal of Biotechnology, **32:** 89-92.

Hamada, H., Sanada, K., Ikeda, S., Oda, T., Williams, H.J., Moyna, G., Scott, A.I., 1996a. Epimerization of taxol® in human cancer cells. Natural Product Letters, **8:**113-117.

Hamada, H., Sanada, K., Furuya, T., Kawabe, S., Jaziri, M., 1996b. Biotransformation of paclitaxel by cell suspension cultures of *Eucalyptus perriniana*. Natural Product Letters, **9:**47-52.

Hirata, T., Ikeda, Y., Izumi, S., Shimoda, K., Hamada, H., Kawamura, T., 1994. Introduction of oxygenated functional groups into monoterpene hydrocarbons with the plant cultured cells. Phytochemistry, **37:** 401-403.

Katoh, K., Ishikawa, M., Miyake, K., Ohta, Y., Hirose, Y., Iwamura, T., 1980. Nutrient utilization and requirement under photoheterotrophic growth of Marchantia polymorpha : Improvement of the culture medium. Physiol. Plant, **49:** 241-247.

Monsarrat, B., Mariel, E., Cros, S., Garés, M., Guénard, D., Guéritte-Voegelein, F., Wright, M., 1990. Taxol metabolism. Isolation and identification of three major metabolites of taxol in rat bile. Drug. Metab. Dispos., **18:** 895-901.

Nakamura, K., Miyoshi, H., Sugiyama, T., Hamada, H., 1995. Diastereo- and enantioselective reduction of ethyl 2-methyl-3-oxobutanoate by plant cell suspension cultures. Phytochemistry, **40:** 1419-1420.

Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P., McPhail, A.T., 1971. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc., **93:** 2325-2327.